Clay-Humus Complexes: A Key Driver of Soil Organic Matter Stabilization in Diverse Indian Soil Orders

ABSTRACT

Aims: This study investigates the stabilization mechanisms of soil organic carbon (SOC) within clay-humus complexes across various Indian soil orders, particularly in paddy soils. By employing Fourier-transform infrared (FTIR) spectroscopy and NaOCl oxidation, the research aims to analyze the influence of clay mineral compositions on SOC stability. The outcomes will enhance the understanding of SOC dynamics and guide targeted soil management practices to improve carbon sequestration and promote sustainable agriculture.
Place and Duration of Study: Soil samples were collected from paddy-growing regions in India-Entisol from Nadia (West Bengal), Inceptisol from Sonipat (Haryana), Vertisol from Belgaum (Karnataka), and Alfisol from Davanagere (Karnataka), between June 2019 and July 2020.
Methodology: The study sampled soils from four Indian regions to investigate SOC stabilization. Clay minerals were analyzed using X-ray diffraction (XRD), and clay-humus complexes were isolated from soils to measure total and NaOCl-resistant carbon. Humic acid was extracted, purified, and characterized for its elemental composition and functional groups. FTIR spectroscopy further analyzed humic acids, clay, and clay-humus complexes. Statistical analysis using ANOVA and DMRT was conducted to compare SOC stability across soil types.

Results: XRD analysis revealed that Vertisol and Inceptisol contained significant amounts of smectite, while Alfisol showed kaolinite and Entisol contained chlorite. Vertisol exhibited the highest total carbon (8.12 g kg^{-1}) and stable carbon (3.96 g kg^{-1}), while Alfisol had the lowest values (2.19 and 0.22 g kg^{-1}, respectively). Humic acid analysis indicated that Vertisol had the highest carbon content and acidity, suggesting strong organic stability. FTIR spectra revealed greater aromaticity in Vertisol's humic acid compared to Entisol.

Conclusion: Variations in clay mineralogy influence carbon stability in soils. Vertisol, rich in smectite, shows the highest carbon stability, while Alfisol with kaolinite is less stable. Analyses demonstrate strong mineral interactions and effective carbon stabilization, emphasizing the importance of clay composition in soil carbon sequestration and management for enhanced soil health.

Keywords: [Carbon stability, Clay minerals, Humic acids and FTIR spectroscopy]

1. INTRODUCTION

Soil organic carbon (SOC) is crucial for soil quality and ecosystem health, serving as a key indicator of soil vitality. Its depletion adversely affects soil aggregation, stability, water retention, fertility and biological activity (Rashid *et al*., 2022). SOC dynamics are influenced by management practices, soil type and climate. SOC stabilization occurs through physical protection, primarily via soil aggregates and chemical processes (Lutzow *et al*., 2009) such as adsorption of SOC to mineral surfaces or aqueous complexation of dissolved organics with mineral weathering products. Chemical reluctance associated with the breakdown of complex organic compounds may potentially contribute to the stability of particular SOC compounds. Chemical weathering reactions cause changes in soil mineralogy over time (Lawrence *et al*., 2015) and other soil parameters that influence SOC stability as a result of the evolution of soil mineralogy. Because of soil mineralogy, the relative relevance of various mechanisms of soil organic matter (SOM) physical and chemical protection changes over time. For decades, it has been well-known that SOM is mostly linked to the mineral structure with varied degrees of stability by hydrogen bonds, van der Waals bonds, ligand exchange, and polyvalent cation bridging (Stoner *et al*., 2023; Mabagala, 2022). As a result, it is protected from degradation by various agents. The amount to which carbon (C) is protected from decomposition is largely determined by the mineral makeup of clay particles (Schweizer *et al*., 2021: Yang *et al*., 2021). Clay minerals not only stabilize SOM, but also reduce the risk of SOM loss through respiration, erosion, and leaching. The kind of phyllosilicate clays in the soil, influence the stabilization and subsequent buildup of organic carbon (OC). Due to their charge characteristics, small particle size, and large surface area, clay minerals are very important for organic matter stabilization. However, the extent of protection provided to SOM by interaction with minerals varies as different clay minerals have different specific surface areas and charge characteristics. Because the dominant clay minerals in different soil orders differ, the same management approaches may have distinct effects on C-stabilization by clay minerals in different soil orders. There are various mechanisms involved in the stabilization of OC and different techniques are utilized to isolate the old and chemically resistant OC component from soils. The use of sodium hypochlorite (NaOCl) to create a chemically resistant SOM fraction attached to mineral surfaces (Bottone *et al*. 2022). The OC that is resistant to NaOCl oxidation is referred to as a recalcitrant or stable OC (Jagadamma *et al.* 2010). Following NaOCl treatment, we can extract mineral-protected OC by the dissolution of the mineral phase. We hypothesized that OC left after the NaOCl treatment was largely stabilized by its inherent chemical composition, thus representing recalcitrant or stable OC in soil.

Previous research has investigated the impact of long-term fertilization and manuring with diverse organics on carbon stability in the clay-humus complex across soils with varying clay mineralogy. Distinct clay minerals and bridging cations dominate different soil orders (Ahmed *et al*. 2002). A recent study by Kumar *et al*. (2022) explored the influence of integrated farmyard manure, green manure, and biofertilizers on soil carbon stability in Inceptisol under rice-wheat rotation in semi-arid sub-tropical India. While conservation tillage, particularly with residue conservation, has shown positive effects on soil composition, water-stable aggregates, and microbial biomass in Inceptisols under a rice-wheat cropping system (Naresh *et al*. 2018), there remains a gap in understanding the dynamics in paddy soils-flooded or submerged soils used for rice cultivation, acting as a source and sink of carbon. The carbon status of these soils is influenced by regional mineralogy as well as farmers' management practices. To unravel the mechanisms of OC association with mineral surfaces and its stability, particularly in paddy soils, comprehensive insights into humic substances such as humic acid (HA) and the intricate processes of clay-humus formation are essential. The present study hypothesizes that NaOCl-resistant carbon represents stable soil organic carbon (SOC) that may have stronger chemical bonds with crystalline silicate clay minerals. Thus, considering the importance of SOC in clay-humus complexes, our study was conducted to better understand the FTIR spectroscopy of HA, clay, and clay-humus complexes isolated from diverse soil orders of paddy soils, and to determine the stability of carbon in these complexes through the NaOCl method. The study will contribute to advancing our understanding of carbon stability in different soil orders while enabling targeted strategies to optimize carbon sequestration and support sustainable agriculture.

2. MATERIAL AND METHODS

To address the above objectives, HA, clay and clay-humus complexes were extracted from diverse soil orders and the NaOCl-resistant carbon in the clay-humus complexes was estimated. FTIR spectroscopy was used to characterize the functional groups of HA, clay and clay-humus complexes by analyzing infrared absorption peaks, focusing on carbon stabilization in different soil orders

2.1 Location and Soil Sampling

Soil samples were collected from diverse paddy-growing states in India, representing various soil orders Entisol from Nadia, West Bengal; Inceptisol from Sonipat, Haryana;Vertisol from Belgaum, Karnataka; and Alfisol from Davanagere, Karnataka. Replicated composite soil samples were collected at a depth of 0-30 cm. Soil samples were dried in the air, ground by a wooden pestle and mortar, and passed through a 2 mm sieve before analysis. Vertisol is clay-rich soil found in Belgaum, Karnataka, with high fertility and swelling/shrinking properties. Alfisol is moderately leached, fertile soils found in Davangere, Karnataka. Inceptisol is the young soil with minimal horizon development, found in Sonipat, Haryana, and suitable for agriculture. Entisol is the youngest soil with little horizon development, found in Nadia, West Bengal, with lower fertility compared to other orders (Table 1).

Table 1. Initial properties of soil collected from diverse soil orders of India

Soil orders	Vertisol	Alfisol	Inceptisol	Entisol
Location	Belguam, Karnataka	Davangere, Karnataka	Sonipat, Haryana	Nadia, west Bengal
Climate	Tropical	Tropical	Humid subtropical	Humid Tropical
pH	7.89	6.86	7.84	6.64
OC (g/kg)	9.91	6.82	9.21	3.76
CEC (Cmol(p$^+$)/kg)	28.44	12.27	12.36	8.67
Sand (%)	10.11	55.84	40.48	40.43
Silt (%)	20.09	8.27	25.98	23.09
Clay (%)	70.1	35.9	33.54	36.28
Texture	Clay	Sandy clay loam	Clay loam	Clay loam

2.2 Clay Mineralogy Study

Clay mineralogy of the collected soil was performed by separating the clay particles (< 2 μm) following the procedure outlined by Jackson (1985). The clay samples underwent four different treatments: (i) magnesium (Mg) saturated and air-dried (Mg-air), (ii) Mg-saturated and glycerol solvated (Mg-gly), (iii) potassium (K) saturated and air-dried (K-air), and (iv) K saturated and heated at 550 °C for 2 hours (K-550). The X-ray diffractograms of the treated clay samples were recorded using a Philips diffractometer (X-ray generator: PW-1729, diffractometer control: PW-1710, Philips, Holland), utilizing Ni-filtered Cu-Kα radiation at a scanning speed of 1.5° 2θ min-1.

2.3 Clay-Humus Complex Analysis

The separation of the clay-humus complex from bulk soil was performed using the method described by Datta et al. (2015). In brief, 20 g of soil was mixed with 200 ml of distilled water in a stainless steel beaker and stirred for 15 minutes using a mechanical stirrer. Following this, vibrate the mixture under an ultrasonic vibrator for 5 minutes to further disperse the micro-aggregates. The resulting suspension was transferred to a 2.5L bottle and distilled water was added to fill it to the neck. This suspension was left undisturbed for 8 hours, after which the upper 10 cm, containing the clay-humus complex with a diameter less than 2 μm (as per Stokes' law, Jackson, 1985), was siphoned off. The process was repeated by adding more distilled water to the remaining suspension and allowing it to settle until the upper portion became clear.

The extracted suspensions containing clay-humus complex were pooled together, concentrated through repeated centrifugation and decantation, and then freeze-dried using a lyophilizer (Labconco, USA). The total carbon content of the clay-humus complex is determined using a CHNS analyzer, which measures carbon, hydrogen, nitrogen, and sulfur through dry combustion. NaOCl-resistant C is measured by treating the clay-humus complex with NaOCl to oxidize and remove labile organic matter, leaving behind the stable NaOCl-resistant C and the labile carbon is calculated as the difference between total carbon and NaOCl-resistant carbon, representing the more readily decomposable fraction of organic matter.

2.4 Extraction and Characterization of humic acid (HA)

The extraction HAs from soils of various orders was conducted following the International Humic Substance Society (IHSS) methodology. Initially, air-dried soil samples were equilibrated to a pH of 1-2 using 1 M HCl, with a final solution volume of 10 ml g-1 of soil. The mixture was shaken for 1h and allowed to settle, after which the supernatant was separated for fulvic acid isolation. The soil residue was neutralized with 1 M NaOH to pH 7 and then treated with 0.1 M NaOH for at least 4h, maintaining a 10:1 extractant-to-soil ratio. Following this, the suspension settled overnight and the supernatant was collected and acidified to pH 1 using 6 M HCl while stirring. This mixture stood for 12-16 h to precipitate HA, which was then separated by centrifugation. The HA was redissolved in a minimal volume of 0.1 M KOH under nitrogen and solid KCl was added to achieve a concentration of 0.3 M [K+]. After high speed centrifugation to remove suspended solids HA was re-precipitated by adding 6 M HCl allowing it to stand again for 12-16 h before further centrifugation. Subsequently, the HA precipitate underwent treatment with a solution of 0.1 M HCl/0.3 M HF in a mechanical shaker overnight at room temperature. This process was repeated until the ash content fell below 1%. The precipitate was then placed in a Visking dialysis tube and dialyzed against distilled water until Cl-1 ions were undetectable using AgNO3. Finally, the HA was freeze-dried. For elemental characterization, a CHNS analyzer was utilized along with protocols for determining the E4/E6 ratio, total acidity, carboxylic acid functional groups and phenolic-OH groups as per established methods (Stevenson, 1994; Schnitzer, 1965).

2.5 FTIR Spectroscopy

The stabilization of C was probed by spectroscopic analysis of HA, clay and clay-humus complexes isolated from different soil orders. Samples were vacuum dried, ground, and characterized using a Bruker (Alpha) instrument. FTIR spectra (600-4000 cm-1) of HA, clay, and clay-humus complexes were recorded on KBr-pressed tablets.

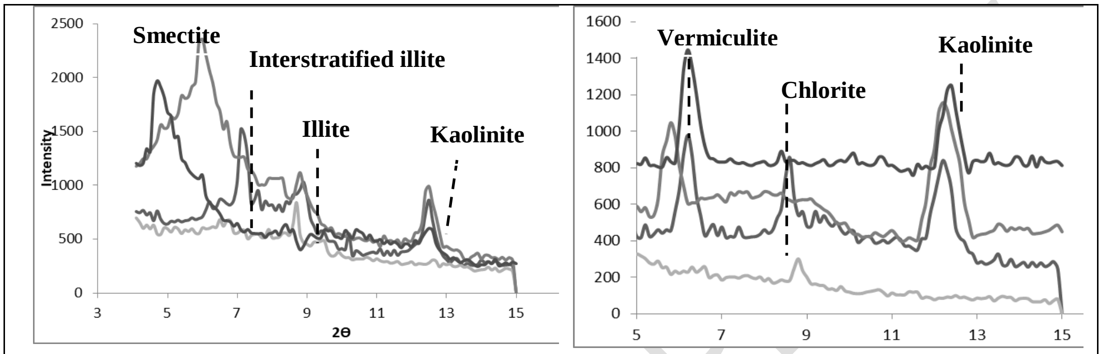
2.6 Statistical Analysis

Data underwent Analysis of Variance (ANOVA), and means were compared using Duncan's Multiple Range Test (DMRT) in Windows-based SPSS version 10.0. This comprehensive methodology ensures a robust investigation into soil properties and organic matter dynamics across diverse soil orders..

3. RESULTS

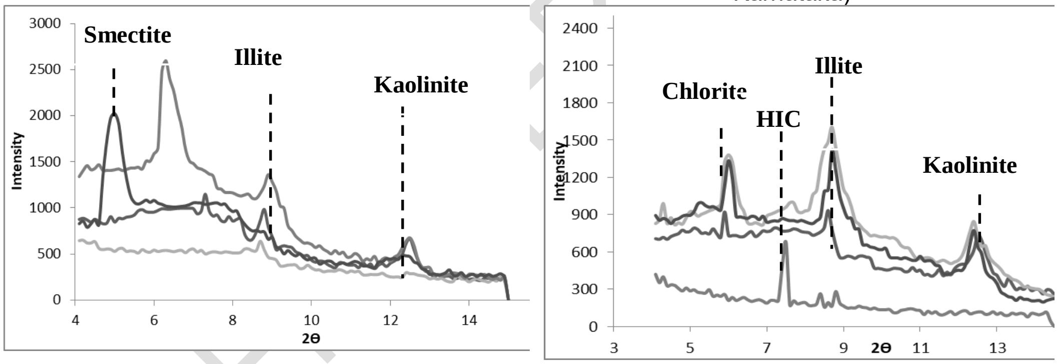
3.1 Clay mineralogy of soils

X-ray diffraction analysis of clay sample from Belgaum, Karnataka, revealed key mineral insights (Fig.1).The basal reflection at 6.1° 2θ (d value 1.43nm) shifted to 4.9° 2θ (d value 1.78) after glycerol treatment, indicating the presence of smectite. Smectite is a significant component in Vertisols, reflecting their Vertic properties. Absorption peaks at 8.5-9° 2θ (d value 1.02-0.97 nm) suggest the presence of Mica clay minerals and interstratified illite is also evident. K-saturated samples showed a peak at 12.5° 2θ (d value 0.70 nm), disappearing at 550°C, confirming the presence of kaolinite clay minerals.



a. XRD pattern of Vertisol (Belgaum, Karnataka)

b. XRD pattern of Alfisol (Davangere, Karnataka)



c. XRD pattern of Inceptisol (Sonipat, Haryana)

d. XRD pattern of Entisol (Nadia, West Bengal)

K-air ■ K 550 ■ Mg air ■ Mg glycerol ■

Fig1. Characterization and Identification of clay mineral in diverse soil orders of India by X-ray diffraction method

XRD analysis of Alfisol soil revealed significant mineral insights (Figure1). Kaolinite was evident in all treated samples, with a distinctive peak at 12.5° 2θ (d value 0.70 nm), which disappeared in K-air at 550°C, indicating thermal degradation. Mg-air samples showed vermiculite with a peak at 5.8° 2θ (d value 1.50 nm), which shifted to 6.2° 2θ (d value 1.40 nm) after Mg-glycerol treatment, suggesting glycerol-induced lattice changes. K-air samples exhibited a persistent chlorite peak at 10° 2θ (d value 0.87 nm) even after heating (K-550), confirming chlorite's resilience to elevated temperatures.

XRD analysis of Inceptisol soil from Sonipat, Haryana (Fig.1), revealed that the Mg-air clay sample showed a peak at 6.1° 2θ (d value 1.43 nm), which shifted to 4.9° 2θ (d value 1.78) after glycerol treatment, indicating the presence of smectite. Peaks at 8.8° 2θ (d value 0.98 nm) suggested illite clay minerals, with no change post-glycerol treatment or heating to 550°C. Absorption peak at 12.1° 2θ (d value 0.72 nm) in untreated and K-550 samples confirmed the presence of kaolinite, which disappeared at 550°C, providing conclusive evidence for kaolinite presence.

3.2 Total and NaOCl-resistant C in clay-humus complexes

The total C content within clay-humus complexes exhibited considerable variability across different soil orders (Table 2), ranging from 2.19 to 8.12 g kg^{-1}. The highest recorded total C content (8.12 g kg^{-1}) was observed in Vertisol, followed by Inceptisol (5.41 g kg^{-1}) and Entisol (3.24 g kg^{-1}), while the lowest was in Alfisol (2.19 g kg^{-1}). Chemically resistant SOM fractions bound to mineral surfaces were obtained through the use of NaOCl method. OC that resists oxidation by NaOCl is considered stable C. The NaOCl-resistant C exhibited significant variation among different soil orders. Vertisol displayed the highest stable C content (3.96 g kg^{-1}), followed by Inceptisol (1.54 g kg^{-1}), with the lowest observed in Alfisol (0.22 g kg^{-1}). The difference between the total C and NaOCl-resistant C values represents the labile C fraction, which ranged from 0.19% in Alfisol to 0.41% in Vertisol.

Table 2. Total C and NaOCl-C content in clay humus complexes of diverse soil orders

Soil Orders	Total C (g kg^{-1})	NaOCl-C (g kg^{-1})	Labile C (%)
Vertisol	8.12^{a}	3.96^{a}	0.41^{a}
Alfisol	2.19^{d}	0.22^{d}	0.19^{d}
Inceptisol	5.41^{b}	1.54^{b}	0.38^{b}
Entisol	3.24^{c}	0.89^{c}	0.21^{c}

3.3 Chemical and spectral characterization of humic acids

Table 3 presents the elemental composition of HA isolated from different soil orders, revealing that HAs from all soil types exhibit a higher carbon content and lower oxygen content, which are indicative of typical HA characteristics. The C content ranged from 53.66 to 57.39 percent, with significantly highest value observed in Vertisol. The sequence of carbon content across soil orders is as follows: Vertisol > Inceptisol > Alfisol > Entisol. Additionally, hydrogen, nitrogen, and sulfur content were significantly higher in Vertisol, followed by Inceptisol, and were lowest in Entisol.

Table 3. Elemental composition of humic acids isolated from diverse soil orders

Soil orders	C (%)	H (%)	N (%)	S (%)	O (%)
Vertisol	57.39^{a}	4.55^{a}	4.64^{a}	0.45^{a}	33.07^{d}
Alfisol	53.77^{c}	3.85^{c}	3.67^{c}	0.30^{c}	38.41^{b}
Inceptisol	54.49^{b}	4.05^{b}	4.01^{b}	0.37^{b}	36.93^{c}
Entisol	53.66^{d}	3.50^{d}	3.25^{d}	0.19^{d}	39.90^{a}

The data on functional groups presented in Table 4 reveal variations in the total acidity (meq g^{-1}) of HAs across different soil orders. The total acidity ranged from 3.80 in Entisol to 6.90 meq. g^{-1}, in Vertisol. Notably, Vertisol exhibited the significantly highest total acidity (6.90 meq. g^{-1}), followed by Inceptisol (6.33 meq. g^{-1}) and Alfisol (5.96 meq. g^{-1}), while Entisol recorded the significantly lowest total acidity (3.80 meq g^{-1}).

Table 4. Acidic Functional groups, total acidity and E4/E6 ratios of humic acids

Soil orders	Total acidity (meq. g^{-1})	Carboxyl group (meq. g^{-1})	Phenolic-OH (meq. g^{-1})	E4/E6 ratio
Vertisol	6.90^{a}	3.85^{a}	3.05^{a}	3.13^{d}
Alfisol	5.96^{c}	3.49^{c}	2.03^{c}	3.96^{b}
Inceptisol	6.33^{b}	3.74^{b}	2.26^{b}	3.59^{c}
Entisol	3.80^{d}	2.40^{d}	1.40^{d}	4.56^{a}

A similar trend was observed in the case of carboxyl and phenolic groups, with Vertisol featuring significantly higher values and Entisol displaying the lowest values. The degree of aliphaticity or aromaticity in humic substances, as indicated by the E4/E6 ratio, exhibited a significantly lower value in Vertisol, followed by Inceptisol, whereas the lowest values were observed in Entisol, followed by Alfisol. The E4/E6 ratio of less than 5 suggests that the HA fraction in these soils possesses a higher degree of condensation of aromatic humic compounds, low acidity, and a high molecular weight. Notably, the higher E4/E6 ratio of 4.56 in Entisol indicates that its HA is less condensed and developed compared to HAs from other soil orders.

3.4 FTIR spectra of humic acids

The FTIR spectra depicted in Fig.2 showcase the distinctive characteristics of HAs isolated from diverse soil orders. The assignments of various absorption peaks are comprehensively detailed in Table 5. Notably, key features are observed in specific spectral regions, including the H-bond OH stretching in the range of 3850-3620 cm^{-1}, aliphatic C-H stretching at 2940-2900 cm^{-1}, and triple bond C≡N and C≡C vibrations spanning 2398-2310 cm^{-1}. Furthermore, significant peaks are identified at 1698-1691 cm^{-1}, indicative of COOH vibrations, and at 1607-1623 cm^{-1}, representing aromatic C=C and C=O stretching in amide, ketone, and quinone groups. The spectral region of 1517-1506 cm^{-1} is associated with COO- symmetric stretching, N-H bending, and C=N stretching. Additionally, the presence of C-O stretching and OH deformation of COOH, along with C-O stretching of aryl ethers, is manifested in the range of 1220-1205 cm^{-1}.

Moreover, distinctive peaks at 1030-950 cm^{-1} are attributed to C-O stretching of polysaccharides and Si-O vibrations arising from silicate impurities. Lastly, aliphatic CH_2 chains are identified in the spectral range of 750-790 cm^{-1}. This detailed analysis provides insights into the molecular composition and functional groups present in HAs from various soil orders, facilitating a comprehensive understanding of their structural characteristics.

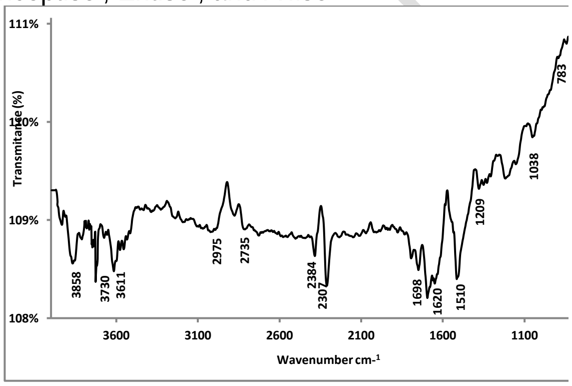
Table 5. Summary of stretching vibrations of various functional groups present in humic acids

Assignment	Vertisol	Alfisol	Inceptisol	Entisol
	(Wavenumber cm^{-1})			
Free OH vibration of residual clay mineral or amine (N-H)	3858,3730	3778,3616	3860, 3735,3615	3872, 3594
Aliphatic C-H stretching	2975,2735	2938	2940,2874	2941

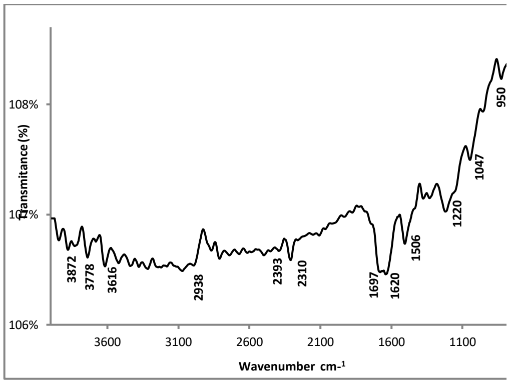
Triple bond C≡N and C≡C	2384,2307	2310	2383	
COOH vibrations	1698	1697	1694	1691
Aromatic C-C, strongly H-bonded C-O of conjugated ketones	1620	1620	1623	1615
COO^- symmetric stretching, N-H bending, C=N stretching (amide II band)	1510	1506	1509	1482
C-O stretching and OH deformation of COOH, C-O stretching of aryl ethers	1208	1220	1201	1232
C-O stretching of polysaccharide and Si-O of silicates impurities	1038	1047	1035	1002
Aliphatic CH_2 chains	783	950	789	812

The FTIR spectra of HAs derived from distinct soil orders exhibit common major absorption peaks with minor variations in their relative intensities. A notable divergence is observed in the spectral features, particularly in the Vertisol and Inceptisol samples, where broad and sharp absorption peaks corresponding to aromatic C-C and strong H-bonded C-O stretching of conjugated ketones (1620-1615 cm^{-1}) are prominent. In contrast, the Entisol and Alfisol spectra reveal distinct and robust COOH vibrations at 1698-1691 cm^{-1}.

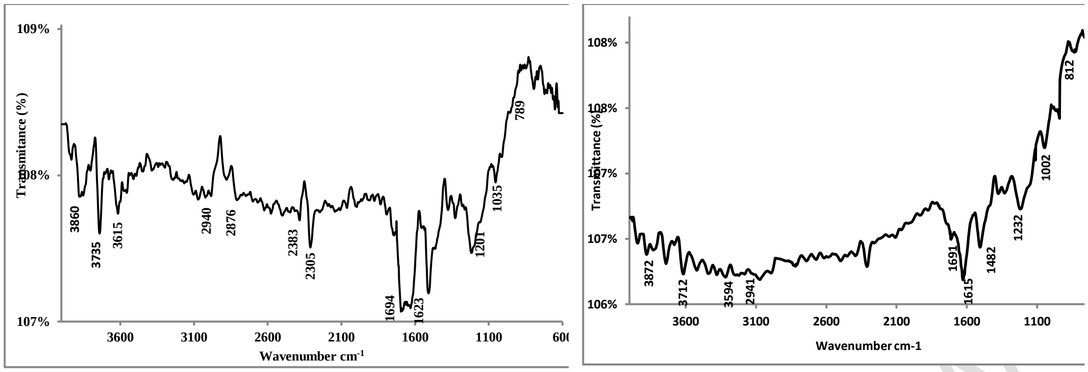
The 3850 to 3420 cm^{-1} region in Vertisol and Inceptisol spectra displays broad absorption peaks attributed to H-bonded OH groups, which are comparatively reduced and weaker in the case of Entisol and Alfisol. Notably, the absorption associated with aliphatic C-H stretching (2940-2900 cm^{-1}) varies significantly, being pronounced in Vertisol and Inceptisol but less prominent in Entisol and Alfisol. Common absorption peaks in the regions 1517-1506 cm^{-1} attributed to COO- symmetric stretching, N-H bending and C=N stretching, at 1220-1205 cm^{-1} attributed to C-O stretching and OH deformation of COOH, around 1030-950 cm^{-1} attributed to C-O stretching of polysaccharide and Si-O vibrations from silicate impurities and around 790-750 cm^{-1} attributed to aliphatic CH_2 chains are discernible in the FTIR spectra of Vertisol, Inceptisol, Entisol, and Alfisol.



a.Vertisol humic acid



b. Alfisol humic acid



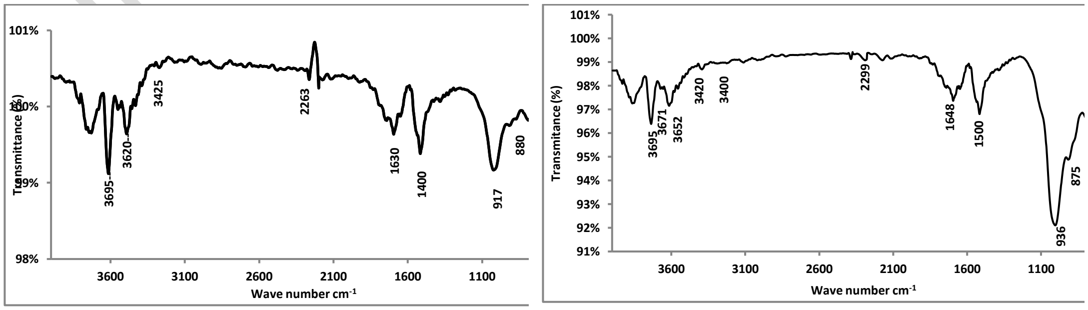
c. Inceptisol humic acid d. Entisol humic acid

Fig2. FTIR spectra of humic acids isolated from soils of diverse soil orders of India

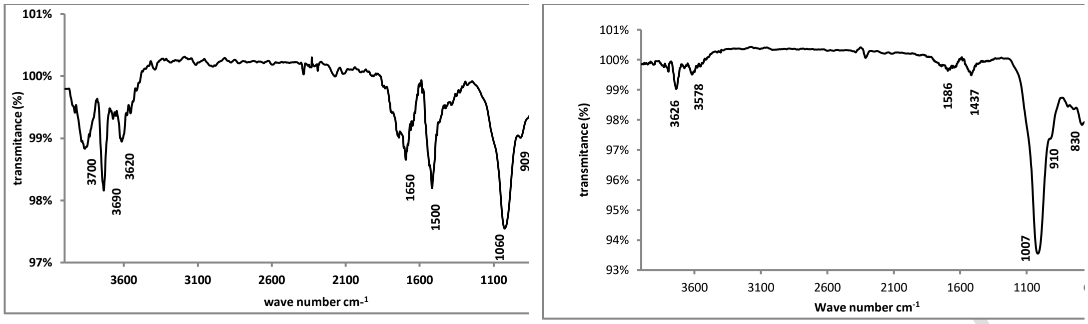
3.5 FTIR spectra of clay

Fig.3 displays the FTIR spectra of clays extracted from diverse soil orders, portraying typical infrared signatures representative of clay minerals. The observed spectra exhibit distinctive features encapsulating three primary regions inherent to clay compositions. Firstly, the stretching and bending vibrations of the inner surface hydroxyl (OH) groups are discernible in the spectral range of 3700 to 3400 cm^{-1}. Secondly, the stretching and bending vibrations associated with the Si-O groups manifest within the region spanning 1000 to 936 cm^{-1}. This region provides insights into the structural characteristics of the clay minerals, particularly in relation to the silicon-oxygen bonding within their lattice framework. Thirdly, the Si-O-M (M = Al, Mg, etc.) vibrations are evident in the spectral range extending from 800 to 600 cm^{-1}. These vibrations elucidate the interactions involving silicon and oxygen with additional elements such as aluminum (Al) or magnesium (Mg), contributing to the overall composition and stability of the clay minerals. The collective analysis of these three prominent regions in the FTIR spectra allows for a comprehensive understanding of the molecular structure and bonding interactions present in clays derived from varying soil orders.

The FTIR spectra of clays derived from Vertisol, Inceptisol, and Entisol reveal distinctive features, particularly two sharp absorption peaks at 3620 and 3697 cm^{-1} in the O-H stretching region. The presence of these peaks indicates the occurrence of smectite clay minerals. Moreover, a stronger intensity of the band stretching at 3620 cm^{-1} compared to 3697 cm^{-1} suggests the prevalence of a substantial amount of 2:1 type clay mineral. In Alfisol, the manifestation of peaks at 3425 cm^{-1} and 1630 cm^{-1}, in addition to the characteristic of smectite peaks, further confirms the presence of smectite.



a. Vertisol clay b. Alfisol clay



c. Inceptisol clay d.Entisol clay

Fig 3. FTIR spectra of clay isolated from soils of diverse soil orders of India

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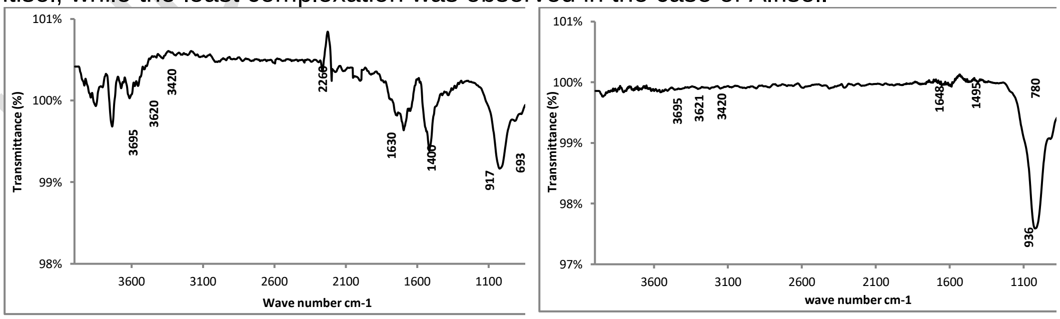
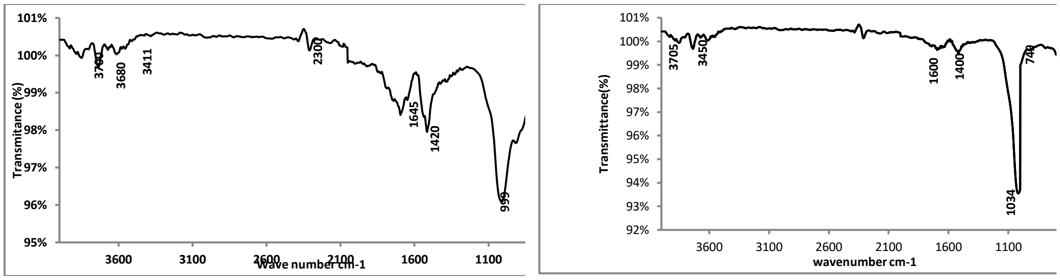
The unique pattern in the Alfisol IR spectra is characterized by four distinct peaks at 3652, 3671, 3694, and 3620 cm⁻¹. These peaks are attributed to vibrations of surface and inner hydroxyl groups, signifying kaolinite as the major clay mineral in Alfisol. Peaks in the range of 1694-1643 cm⁻¹ correspond to strong H-O-H bonds of absorbed water, highlighting the presence of hydrated phyllosilicate minerals such as chlorite, illite, and smectite in Vertisol, Inceptisol, and Entisol. In contrast, in Alfisol, a strong hydrogen-bonded O-H group is observed, indicating the predominance of kaolinite-rich minerals. The stretching and bending vibrations of the Si-O group, within the range of 1000 to 936 cm⁻¹, reveal intense Si-O peaks at 1060 and 1007 cm⁻¹ for Inceptisol and Entisol, respectively. Additionally, a shoulder peak arising from the Al-OH-Al bending vibration is evident around 909 to 936 cm⁻¹ for smectite, illite, and kaolinite, prominently visible in Vertisol and Alfisol, while appearing weakly in Entisol and Inceptisol. This detailed analysis provides insights into the mineralogical composition and structural characteristics of clays across diverse soil orders.

3.6 FTIR spectra of clay-human complex

The FTIR spectra of clay and HA were compared, revealing a noteworthy diminution and attenuation in the clay-humus complex spectra (Fig.4). This attenuation signifies a robust association between HA and clay minerals. The complexation primarily involves the electrostatic, van der Waals, or hydrogen bonding interactions between octahedral hydroxyl (OH) groups in clay and OH groups in HA phenol/amine, resulting in a reduction and shift towards lower frequencies in the clay-humus complex FTIR spectra, particularly in the 3800-3000 cm^{-1} region. This phenomenon was notably pronounced in Vertisol, Inceptisol, and Entisol, while the least complexation was observed in the case of Alfisol.



a. Vertisol clay-humus complex b. Alfisol clay-humus complex



c. Inceptisol clay-humus complex d. Entisol clay-humus complex

Fig4. FTIR spectra of clay-humus complexes isolated from soils of diverse soil orders of India

In all soil orders, specific peaks in the clay and HA spectra, such as those at 2300 cm^{-1}, 2260 cm^{-1}, 2290 cm^{-1}, 1500 cm^{-1}, and 1400 cm^{-1}, exhibited a decrease in intensity and broadening in the clay-humus complexes. This alteration is attributed to the polymerization between clay and HA, indicating the formation of strong associations. Additionally, the peaks in the 1648-1630 cm^{-1} range, assigned to the strong hydrogen-oxygen-hydrogen (H-O-H) bonds of absorbed water in hydrated phyllosilicate minerals, as well as aromatic C=C and C=O stretching in amide, ketone, and quinone groups of HAs, were strongly complexed and exhibited reduced intensity in Vertisol, Inceptisol, and Entisol. Conversely, in Alfisol, the strong hydrogen bonds of kaolinite-rich minerals remained unchanged, while the 1600 cm^{-1} peak in HA samples weakened after complexation in the clay-humus complex. This weakening indicates the involvement of ketones, carboxyl, and amide moieties in complexation with octahedral OH, while the unchanged 1600 cm^{-1} peak suggests that the strongly hydrogen-bonded O-H in the kaolinite-rich layer structure did not participate in complexation.

Furthermore, in the clay-humus complexes of all soil orders, the peak around 1080 to 917 cm^{-1}, associated with inner sphere aluminum hydroxyl (Al-OH) groups, exhibited a reduction, implying the active participation of organic moieties in the complexation process.

4. DISCUSSION

4.1 Stability of C in clay-humus complexes of different soil orders

The stability of C within clay-humus complexes is a crucial aspect of understanding SOC dynamics across different soil orders. The total C content and NaOCl-resistant C enrichment serve as vital indicators, reflecting the persistence of SOC (Das *et al*., 2019). The observed variations in these features among different soil orders (Vertisol, Alfisol, Inceptisol, and Entisol) have been elucidated through a scientific lens. The elevated enrichment of NaOCl-resistant C in Vertisol, compared to other soil orders, may be attributed to the specific mechanisms of carbon protection facilitated by the presence of clay minerals in these soils (Das *et al*., 2019, Ahmed *et al*., 2002). Vertisol, characterized by a higher content of smectite, hydroxyl-interlayered minerals (HIMs), and smectite-rich interstratified clay minerals, exhibits a strong affinity for soil humus (Das *et al*., 2023, Jadhav *et al*., 2023). This affinity is corroborated by X-ray diffractogram analyses revealing the prevalence of these clay minerals in Vertisol. Studies by (Chotzen 2016, Kome *et al*. 2019) have previously noted the tendency of smectite-containing soils and sediments to harbor elevated organic matter. The permanence of negatively charged sites on smectite-rich minerals, resulting from isomorphic substitution, creates abundant active sites on clay mineral surfaces (Huang *et al*., 2024; Kumari and Mohan 2021). This phenomenon enhances the adsorption capacity of clay minerals for organic compounds, contributing to the higher total C and NaOCl-resistant C as observed in Vertisol. Conversely, the lower NaOCl-resistant C enrichment in Alfisol can be attributed to the predominance of kaolinite-rich minerals in these soils (Mikhail and James, 2015, Khan *et al*., 2023). Kaolinite is characterized by lower total surface area, minimal isomorphic substitution, and inaccessible interlayers for foreign molecules, which hinders the humification of organic substances (Kumari and Mohan, 2021, Khan *et al*., 2023). As a result, in the present study, the total C and NaOCl-resistant C content in Alfisol is relatively low compared to other soil orders, signifying a lower C stability within the clay-humus complex. Similar findings were reported by Sukumaran *et al*., 2024, Purakayastha *et al*., 2019, Srinivasarao *et al*., 2009, Ahmed *et al*., 2002.

In Inceptisol and Entisol, the relatively higher NaOCl-resistant C content aligns with the prevalence of illite and illite-rich minerals. These minerals exhibit higher surface area and amphoteric properties on broken edges of clay minerals, facilitating greater retention of organic carbon (Kumari and Mohan, 2021; Khan *et al*., 2023). The observations presented by Wiseman and Puttmann, 2006 support the notion that illite-rich minerals contribute to enhanced C stability in these soil orders. These also align with previous findings by Barre *et al*. (2008), Datta *et al*. (2015), and Das *et al*. (2019

4.2 Elemental and Functional characterization of humic acids

The reactivity of humic compounds in soil is usefully shown by its elemental composition. The most durable and widely dispersed organic material on the earth's surface is humic substances (Lehmann and Kleber, 2015). The elemental composition of HA extracted from Vertisol recorded significantly higher values in the present study, due to intensive humification, a greater degree of condensation of aromatic rings in Vertisol led to greater elemental carbon, nitrogen, and sulphur in humic materials than other soil orders (Tamfuh, 2011; Srinivasarao *et al*. (2009). Similar findings of Krishna and Mohan, 2017; Giweta, 2020 and Zhou *et al*., 2023 stated that the nature of OM (leaf litter, plant residues, FYM, organic manures etc.) added to the soil and its decomposition under various climates (dry and transitional heavy rainfall zones) has a significant influence on the elemental composition of humic substances. The index of functional groups (carboxylic and phenolic-OH groups) expressed as total acidity was significantly greater in Vertisol compared to Inceptisol, Alfisol and Entisol. Tamfuh (2011) and Srinivasarao *et al.* (2009) also observed similar results, attributing the variations in total acidity to inherent differences in the chemical composition and molecular weights of HAs. The E4/E6 ratio was significantly lower in Vertisol while higher in Entisol (Ahmed *et al*., 2002). Generally, the HAs with a ratio of < 5 serve as an index of humification and the ratio narrows with increasing molecular weight and condensation (Srilatha *et al*., 2013). Greater values of the E4/E6 ratio indicate low molecular weight, a low degree of aromatization, and a relatively large proportion of aliphatic structures (Mathew *et al*., 2021; Jadhav *et al*., 2023). This is true for Entisol, where time is a limiting factor (Satisha and Devarajan, 2011; Tahiri *et al*., 2016).

4.3 FTIR spectroscopy of humic acid, clay and clay-humus complexes

HAs are probably the biggest single SOM pool in mineral soils (Banach-Szott and Tobiasova, 2021). The spectral characterization of HA, could reveal the importance of HA in carbon stability in different soil orders. Comparing all the HA and clay IR spectra of different soil orders, the clay-humus complexes IR spectra are reduced and have lower peaks (Ampee, 2018). This indicates that strong bonding between humic substances and the layer silicate minerals (Ampee, 2018; Nuzzo *et al*., 2020). Vertisol and Inceptisol's HA, clay and clay humus complex spectra have similar absorption peaks. The HA spectra of these soil orders have intense and sharp peaks of aromatic C=C and C=O in amide, ketone, and quinone stretching and reduced and weak peaks of aliphatic C-H stretching which indicates that a higher percentage of aromatic C due to the advanced stage of humification, the concentration of recalcitrant organic compounds increased and tends toward higher aromaticity (Giovanela *et al.*, 2010), Suman *et al.*,2020). Absorption caused by aliphatic C-H stretching varied considerably, being strong in Vertisol, Inceptisol but not in Entisol and Alfisol (Ampee, 2018). The absence of pronounced peaks in this region may be due to the extensive substitution of aromatic ring or masking from the broad band resulting from OH stretching (Giovanela *et al.,*2010*,* Zhang *et al.*,2016). This can also be concluded with the support of the IR spectra of clay. In IR spectra of Vertisol and Inceptisol indicated the dominance of smectite and smectite rich minerals which have greater surface area for adsorption and higher layer charge than other silicate layer minerals this led the way for higher affinity towards the organic matter (Sukumaran *et al*. 2024, Das *et al*., 2019). The organic matter fixed within the interlayer of these hydrated phyllosilicates represented the stable carbon resistant to degradation and underwent a humification process which showed higher aromaticity in these soil orders (Xu *et al*., 2024). In IR spectra of Vertisol and Inceptisol clay samples, the peak around 1600 cm^{-1} remains unchanged but in HA samples became weak after complexation. This indicates the involvement of aromatic C=C and C=O in ketones, carboxyl and amide moieties in complexation with octahedral OH which led to produced weak and reduced peaks but the unchanged peaks indicated strong hydrogen bonded O-H in silicate clay layer structure which did not participate in complexation this is true in case of Entisol and Alfisol who has weak and reduced peaks in HAs spectra and the clay mineralogy of Entisol mainly depends on the soil parent materials (Ahmed *et al*., 2002; Das *et al*., 2019; Ampee, 2018)). As the IR spectra indicated the presence of illite, kaolinite, and smectite type clay minerals which show an average stability of C in these soils as compared to Alfisol (Ahmed *et al.,* 2002). From IR spectra of Alfisol clay showed the presences of kaolinite and kaolinite rich minerals which has a lower total surface area and its interlayers are not accessible by foreign molecules, hence very slow humification process occurs in these soil orders because of this reason the Alfisol HA spectra has very low features. The same can be proved by the IR spectra of Alfisol clay humus complex, where the clay-HA linkage is probably through hydrogen or other monovalent cation bridges which is generally very weak in nature causing lower carbon stability (Das *et al*., 2019). Ahmed *et al*. (2002) also reported that Alfisol has weakest clay-HA bond compared to Vertisol, Mollisol and Entisol.

5. CONCLUSIONS

Variations in clay mineralogy among soil types significantly influence carbon stability in clay-humus complexes. Vertisol, rich in smectite, exhibits the highest carbon stability, while Alfisol, dominated by kaolinite, shows lower stability. The content of NaOCl-resistant carbon reflects this trend, underscoring the role of clay minerals in carbon sequestration. Elemental and functional analyses of HAs support these findings, with Vertisol demonstrating advanced humification. FTIR spectroscopy highlights strong complexation in Vertisol, Inceptisol, and Entisol, emphasizing effective carbon stabilization through mineral interactions, especially in soils with higher smectite content. This study underscores the cruci al role of soil mineralogy, particularly clay composition, in influencing carbon stability across different soil orders. The insights gained from mineralogical, elemental, and spectroscopic analyses enhance our understanding of SOC dynamics, providing a scientific basis for soil management practices aimed at improving carbon sequestration and overall soil health.

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